Remarks

Claims 1-15, 23, 25 and 32 are pending. Claims 1-15, 23, 25 and 32 have been rejected. In the present response, claims 1, 2, 4-6, 8 and 32 have been amended and claims 3, 9, 12, 23 and 25 have been canceled without prejudice. Support for the amended claims is found throughout the specification as described in the following remarks. No new matter has been added by way of this amendment. Reconsideration and withdrawal of the rejections are respectfully requested in light of these amendments and the following remarks.

35 U.S.C. § 112, Second Paragraph

The Examiner has maintained the rejection of claims 1 (claims 3-6, 11 and 13-14 dependent therefrom), 2, 7-10, 12, 15, 23, 25 and rejected new claim 32 as being indefinite in the recitation of "cdk5", "cdk5 activity" and "Dab1".

The Examiner maintains that it is unclear as to the scope of proteins that are intended as being encompassed by the terms "Cdk5" and "Dab1" and what activity or activities is/are encompassed by the term "Cdk5 activity." The Examiner asserts that the specification fails to define which of the many, well known properties of "Cdk5" or "Dab1" protein are necessary for inclusion of a cyclin-dependent kinase or a disabled-1 protein to distinguish it from similar proteins that share these characteristics.

Applicants respectfully traverse the rejection. Claim 1 has been amended to indicate that "Cdk5 activity" as taught by the present invention is "Cdk5 serine kinase activity". Support for this amendment is found throughout the specification, particularly on page 4, lines 14 – 21. Claim 1 has also been amended to demonstrate that the serine phosphorylated by Cdk5 kinase activity of the present invention is "selected from the group consisting of a serine corresponding to position 491 of SEQ ID NO:4 and a serine corresponding to position 515 of SEQ ID NO:4". Support for this amendment can be found on page 8, lines 8 – 13 and the paragraph spanning from page 20 line 8 through page 21, line 2.

A patent need not teach, and preferably omits, what is well known in the art. *Hybridtech, Inc. v. Monoclonal Antibodies, Inc.* 231 USPQ 81, 94 (Fed. Cir. 1986)(citing

Lindemann Maschinenfabrik v. American Hoist and Derrick, 221 USPQ 481, 489 (Fed. Cir. 1984); *Stahelin v. Secher,* 24 USPQ2d 1513, 1516 (Bd. Pat. App. & Int. 1992). Publications using the terms Cdk5 and Dab1 prior to the filing of the present invention reveal that these terms were familiar and well understood by those of skill in the art at the time the present application was filed and could be distinguished from other related proteins, such as other Cdk and Dab proteins, according to their characteristic features. Based on the prior art, a skilled artisan would be able to routinely identify any Cdk5 protein that has the Cdk5 serine kinase activity of the present invention. Furthermore, one skilled in the art would be able to identify a Dab1 protein within a biological sample with the structural properties of the claimed invention, i.e. a serine at the 491 or 515 positions.

The accompanying expert declaration of Dr. Thomas Curran, a co-inventor of the present application and an expert in the field of developmental neurobiology, supports Applicants' position that "Cdk5", "Dab1" and "Cdk5 serine kinase activity" were well known terms in the art at the time the present invention was filed and that one of skill in the art would recognize the meaning of these terms.

Applicants have also included an alignment of the mouse, rat and human Cdk5 and Dab1 proteins (Exhibits A and B). The alignments show that both proteins are highly conserved among the three species. Furthermore, Dab1 protein from all three species contains a serine at position 491 and 515. This is further evidence that Cdk5 and Dab1 proteins are easily distinguishable from other related proteins by those of skill in the art.

The present invention is based on the discovery that Dab1 is specifically phosphorylated by Cdk5 and that this phosphorylation can be used as a marker for Cdk5 serine kinase activity. One cannot directly determine the amount of Cdk5 kinase activity based on the levels of Cdk5 protein since Cdk5 kinase activity is dependent on the Cdk5 protein binding to a regulatory subunit. Thus, the present invention provides a unique method for determining Cdk5 serine kinase activity. Applicants have conducted experiments in the mouse and fully expect the same functional relationship between Cdk5 and Dab1 found in the mouse to exist in all other organisms that contain Cdk5 and Dab1. Consistent with this expectation, the abstract by Wang et al. presented at the Society for

Neuroscience meeting in November, 2003 (abstract provided as Exhibit C), shows that this relationship also holds true in the rat.

Thus, the Claims of the present invention relate to any Cdk5 protein (1) that has serine kinase activity, (2) that phosphorylates Dab1 protein within the same biological sample, (3) and that phosphorylates the Dab1 protein on a serine corresponding to serine 419 or 515 of SEQ ID NO:4. Applicants have shown that the terms "Cdk5", "Dab1" and "Cdk5 serine kinase activity" are well known in the prior art, especially in the context of the present invention. Applicants have shown that the present invention is based upon a general relationship between Cdk5 and Dab1 proteins and should not be restricted to a particular Cdk5 or Dab1 of a single species. Applicants therefore respectfully request reconsideration and withdrawal of this rejection as applied to the remaining pending claims. Should this rejection be maintained, Applicants ask that the Examiner provide a scientific rationale or evidentiary support for his assertion that the Cdk5/Dab1 relationship which forms the basis of the invention is peculiar to a single species and not shared by Cdk5/Dab1 proteins from other species.

The Examiner also asserts that it is unclear whether the terms are meant to encompass those Cdk5's or Dab1's that are considered to be "naturally-occurring" or to also encompass those that are mutants and/or fragments of a known Cdk5 or Dab1 protein.

Applicants respectfully disagree. Claim 1 states that the method is one for determining Cdk5 kinase activity *in a biological sample* and the Dab1 protein is a "Dab1 *in said sample*" (emphasis added). If a Dab1 protein within a particular biological sample is phosphorylated on a serine that corresponds to serine 419 or 515 of SEQ ID NO:4 this phosphorylation shows that the Cdk5 which is found in the biological sample has Cdk5 serine kinase activity and is encompassed by the scope of the claim. Applicants have shown that Cdk5 and Dab1 are well known terms in the art and have provided methods for determining if a Dab1 within a sample is phosphorylated on a serine corresponding to 491 of SEQ ID NO:4. Therefore, if mutants and/or fragments of known Cdk5 or Dab1 found within a biological sample exhibit the properties described in the claims, such mutants and/or fragments are encompassed by the present invention.

The Examiner also maintained the rejection of claims 1 (claims 2, 4-9, 11-15, 23 and 25 dependent therefrom), 10 (claims 12-15 dependent therefrom), and rejected new claim 32 as being indefinite in the recitation of "a candidate sequence preferred by Cdk5 activity". The Examiner asserts that it is unclear from the specification and claims as to whether all sequences that have a serine followed by proline at the +1 position and a lysine in the +3 position are those that are "preferred" by a "Cdk5 activity" or whether only a subset of those sequences are meant to be encompassed.

Applicants have amended claim 1 to show that the serine encompassed by the scope of the claims is a serine selected from the group consisting of a serine corresponding to position 491 of SEQ ID NO:4 and a serine corresponding to position 515 of SEQ ID NO:4 and have removed the phrase "a candidate sequence preferred by Cdk5 activity . Therefore any perceived indefiniteness attributed to this phrase has been removed from the claims. Reconsideration and withdrawal of this rejection as applied to the remaining pending claims are respectfully requested.

The Examiner also maintained the rejection of claims 4-6 as being unclear in the recitation of "derived from" and has suggested replacement with “isolated from”. Applicants have adopted this suggestion since the meaning of “isolated from” according to the Examiner is consistent with Applicants' intended meaning for “derived from” (i.e. "to get or obtain something from something else" and "tissues, blood, etc. isolated directly from a subject as well as other substances… which are derived from the directly isolated tissues"; see page 5, #[8] through page 6, of the Office Action dated 01/26/2005). Reconsideration and withdrawal of this rejection in view of this amendment are respectfully requested.

Claim 2 was also rejected as being indefinite because of the recitation of "murine Dab1". Applicants have now identified the murine Dab1 as having SEQ ID NO:4. Support for incorporation of SEQ ID NO:4 in the specification can be found on page 4, lines 22-25, which identifies the genbank accession number of murine Dab1. SEQ ID NO: 4 was extracted from the sequence information referenced under this genbank accession number. Reconsideration and withdrawal of this rejection are respectfully requested.

Claim 2 was rejected as being indefinite in the recitation of "the candidate sequence is included within the Dab1 amino acids selected from...". Applicants have amended Claim 2 to delete recitation of this phrase, thus rendering this rejection moot. Reconsideration and withdrawal of this rejection are respectfully requested.

The Examiner rejected Claim 12 as unclear in the recitation of "serine contained within the amino acids... of SEQ ID NO:1 and SEQ ID NO:2" as it is unclear as to which serine is being phosphorylated in each sequence. Applicants have canceled Claim 12, thus rendering this rejection moot.

The Examiner rejected Claim 32 as being indefinite in the recitation of "GenBank accession number 1771281". Applicants have incorporated SEQ ID NO:4, which is the mouse Dab1 sequence found in GenBank accession number 1771281 on February 19, 2002, the filing date of the present application. Reconsideration and withdrawal of this rejection are respectfully requested.

35 USC § 101

The Examiner maintained the rejection of Claims 23 and 25 under 35 U.S.C. 101 for lack of sufficient guidance in the specification or the prior art for using the methods of the claims for detecting neurological disorders. The Examiner maintains that further experimentation is required to identify a "real world" use for the claimed invention.

Applicants disagree and note that an incomplete or imperfect association between Cdk5 activity and the presence of a neurological disorder is sufficient to provide a utility for the claimed method. However, in the interest of furthering prosecution, Applicants have canceled Claims 23 and 25 without prejudice, reserving the right to pursue the subject matter of these claims in a further continuation or divisional application. Applicants note cancellation of these claims does not represent surrender of the covered subject matter since the scope of the remaining pending claims of the present application encompass the methods of canceled claims 23 and 25.

35 USC § 112, First Paragraph Claim Rejections

The Examiner rejected Claims 1-15, 23, 25 and 32 under 35 USC 112, first paragraph, as failing to comply with the written description requirement. The Examiner maintains that the rejection is based upon the failure of the disclosed representative species (three for Cdk5 and the two for Dab1) to describe all members of the respective genus, which encompasses species that are widely variant, particularly in view of the indefiniteness of the terms "Cdk5" and "Dab1". The Examiner maintains that there is no disclosed structure-function correlation among Cdk5 or Dab1 polypeptides in the specification or prior art. As such, applicants have failed to adequately describe the recited genus of Cdk5 or Dab1 polypeptides, which encompasses widely variant species.

Applicants respectfully disagree. Applicants have amended Claim 1 to recite "a serine selected from the group consisting of a serine corresponding to position 491 of SEQ ID NO:4 and a serine corresponding to position 515 of SEQ ID NO:4". Applicants have shown by expert declaration that "Cdk5" and "Dab1" are well known in the art. Cdk5 is easily distinguished from other closely related cyclin dependent kinases, such as Cdk4 and Cdk6, by those skilled in the art. The same holds true for distinguishing Dab1 from the closely related Dab2 protein. Furthermore, Applicants have shown that the sequences for the Cdk5 and Dab1 species identified in the present application (mouse, rat and human) are highly conserved. Applicants maintain that the terms "Cdk5" and "Dab1" are definite. One of skill in the art is capable of recognizing a Dab1 protein in a biological sample and determining whether it is phosphorylated on a serine corresponding to position 491 or 515 of SEQ ID NO:4 by a Cdk5 in the same biological sample. Reconsideration and withdrawal of this rejection are requested.

With regard to the genus of recited antibodies, the Examiner maintains that the genus of recited antibodies is not limited to those that bind to a "candidate sequence" of Cdk5, but is a genus of antibodies that binds to a phosphorylated or unphosphorylated Dab1 polypeptide.

Applicants respectfully disagree with the rejection. Applicants have amended Claim 8 to more distinctly show that an antibody used prior to the determining step may be an antibody that binds to Dab1, whether it is phosphorylated or unphosphorylated on said serine. For example, any Dab1 antibody that binds to Dab1 may be used for

immunoprecipitation. Such a procedure is normally performed to separate a particular protein from other proteins in a sample so that the particular protein may be studied further. An example of immunoprecipitation with an anti-Dab1 antibody prior to the determining step can be found in the Examples on page 18, lines 3 – 7.

Claim 10 refers to an antibody used in the determining step which binds to Dab1 only when it is phosphorylated on a serine corresponding to serine 491 or 515 of SEQ ID NO:4.

Applicants have canceled Claim 9, rendering this rejection moot.

Sufficient Guidance and Working Examples

The Examiner maintains the rejection of Claims 1-15, 23, 25 and 32 under 35 USC 112. The Examiner maintains that the specification does not provide enablement for the broad scope of the claimed methods. The Examiner asserts that the alleged novel relationship claimed by Applicants, i.e., the phosphorylation of Dab1 by Cdk5, has not been shown in all organisms that express "Cdk5" and "Dab1" polypeptides. The Examiner asserts that due to structural differences that are likely to occur among different organisms, it is unclear as to whether any Cdk5 from any organism will phosphorylate any Dab1 from any other species. Furthermore, the claims encompass mutant and variant Cdk5 and Dab1 polypeptides. However, the Examiner has not provided any evidence to support such unpredictability, but asserts that one skilled in the art would recognize such unpredictability, particularly as the method is dependent upon a biological relationship. The Examiner asserts that arguments of counsel alone cannot take the place of evidence. The Examiner maintains that regarding Claims 23 and 25, no working examples for detecting a neurological disorder have been disclosed and invites Applicants to direct the Examiner's attention to such a working example.

Applicants traverse the rejection. Applicants have provided sequence alignments for mouse, rat and human Cdk5 and Dab1 proteins, which show that both proteins are highly conserved among the species. Furthermore, these Dab1 sequences all contain serines at positions 491 and 515. An example was provided in the specification showing that the claimed invention works in the mouse. Furthermore, Applicants have provided an

abstract by Wang et al. presented at the Society for Neuroscience meeting in November, 2003, showing the claimed invention works in the rat. Applicants have provided evidence that one skilled in the art can identify a Cdk5 and Dab1 protein within a biological sample from two different species and that one skilled in the art can identify Cdk5 kinase activity based upon the phosphorylation of a serine corresponding to 491 or 515 of SEQ ID NO:4. Again, Applicants respectfully request that the Examiner provide evidence to support the contention that the demonstrated relationship between Cdk5 activity and Dab1 serine phosphorylation in the mouse (and now rat) would not be consistent in other species.

As for Claims 23 and 25, Applicants have canceled these claims. However, Applicants maintain that the scope of the remaining pending claims encompass the methods of the canceled claims. In response to the Examiner's request for a working example, Applicants have provided an abstract by Wang et al. describing experiments in which rat Dab1 was phosphorylated on serine 491 in response to ischemia-reperfusion. The authors concluded that increased Dab1 phosphorylation was observed after middle cerebral artery occlusion, supporting the association of Cdk5 activity with stroke pathology.

High Degree of Predictability

The Examiner maintains that it is unclear as to the scope of proteins that are considered to be "Cdk5" or "Dab1" polypeptides and it is unclear as to those "preferred" candidate sequences, particularly in view of the indefiniteness of the terms. The Examiner maintains that it is highly unpredictable as to whether a "Cdk5" from any source, including mutants and variants thereof, will phosphorylate a "Dab1" from any source, including mutants and variants thereof. The Examiner also maintains that it is highly unpredictable as to whether phosphorylated Dab1 from any source is related only to Cdk5 phosphorylation as it is likely that Dab1 is phosphorylated by other kinases in other organisms. Regarding Claims 23 and 25, the Examiner asserts that it is highly unpredictable as to whether increased Cdk5 activity is associated with *any* neurodegenerative disorder. The Examiner asserts that the specification fails to provide

even a single working example and/or guidance for detecting even a single neurodegenerative disorder and invites Applicants to direct the Examiner's attention to such a working example.

Applicants respectfully disagree. The scope of the claims is limited to determining the Cdk5 serine kinase activity in *a biological sample* by determining whether or not Dab1 from *the biological sample* is phosphorylated on a serine corresponding to position 491 or 515 of SEQ ID NO:4.

As for the Examiner's rejection based on unpredictability based on whether phosphorylated Dab1 is related only to Cdk5 phosphorylation, applicants show on page 21, lines 24 – 31 that Dab1 was not phosphorylated at serine 491 in Cdk5 knockout mice, showing that in vivo phosphorylation of serine 491 of Dab1 is catalyzed only by Cdk5. Applicants maintain that if any other kinase were responsible for the phosphorylation, serine 491 of Dab1 would be phosphorylated in Cdk5 knockout mice.

As for the rejection as it pertains to Claims 23 and 25, as shown above, increased phosphorylation of Dab1 is observed in rat brains that have experienced ischemia-reperfusion injury, validating Cdk5 activity involvement in stroke pathology. However, to further prosecution, Claims 23 and 25 have been canceled without prejudice.

Amount of Experimentation Required Is Routine

The Examiner maintains that Claims 23 and 25 require an undue amount of experimentation to make and/or use the full scope of the claimed methods. The Examiner states that the specification fails to provide even a single working example for practicing the claimed method and asks Applicants to direct the Examiner's attention to such in the specification. The Examiner asserts that it is not even clear that increased levels of Cdk5 activity can be used to detect a neurological disorder, and even if so, the specification fails to provide the necessary guidance for determining whether one has a neurological disorder based on increased levels, e.g., what level of increased Cdk5 activity as compared to a control is indicative of a neurological disorder, and if one has a neurological disorder, which disorder.

For the reasons stated above, Applicants disagree with this rejection. However, to further prosecution, Applicants have canceled Claims 23 and 25, rending this rejection moot.

In view of the above arguments and amendments, all grounds for the rejection under 35 U.S.C. § 112, first paragraph have been obviated or overcome. Reconsideration and withdrawal of these rejections are respectfully requested.

It is believed that all the rejections have been obviated or overcome and the claims are in condition for allowance.

It is not believed that extensions of time or fees for net addition of claims are required. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 501968.

Respectfully submitted,

Shawn A. Hawkins

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Exhibit A
Cdk5 Protein Alignment
Mouse, Rat and Human

```
MouseCdk5       MQKYEKLEKIGEGTYGTVFKAKNRETHEIVALKRVRLDDDDEGVPSSALREICLLKELKH 60
RatCdk5         MQKYEKLEKIGEGTYGTVFKAKNRETHEIVALKRVRLDDDDEGVPSSALREICLLKELKH 60
HumanCdk5       MQKYEKLEKIGEGTYGTVFKAKNRETHEIVALKRVRLDDDDEGVPSSALREICLLKELKH 60
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MouseCdk5       KNIVRLHDVLHSDKKLTLVFEFCDQDLKKYFDSCNGDLDPEIVKSFLFQLLKGLGFCHSR 120
RatCdk5         KNIVRLHDVLHSDKKLTLVFEFCDQDLKKYFDSCNGDLDPEIVKSLLFQLLKGLGFCHSR 120
HumanCdk5       KNIVRLHDVLHSDKKLTLVFEFCDQDLKKYFDSCNGDLDPEIVKSFLFQLLKGLGFCHSR 120
                ************************************************:***********

MouseCdk5       NVLHRDLKPQNLLINRNGELKLADFGLARAFGIPVRCYSAEVVTLWYRPPDVLFGAKLYS 180
RatCdk5         NVLHRDLKPQNLLINRNGELKLADFGLARAFGIPVRCYSAEVVTLWYRPPDVLFGAKLYS 180
HumanCdk5       NVLHRDLKPQNLLINRNGELKLADFGLARAFGIPVRCYSAEVVTLWYRPPDVLFGAKLYS 180
                ************************************************************

MouseCdk5       TSIDMWSAGCIFAELANAGRPLFPGNDVDDQLKRIFRLLGTPTEEQWPAMTKLPDYKPYP 240
RatCdk5         TSIDMWSAGCIFAELANAGRPLFPGNDVDDQLKRIFRLLGTPTEEQWPAMTKLPDYKPYP 240
HumanCdk5       TSIDMWSAGCIFAELANAGRPLFPGNDVDDQLKRIFRLLGTPTEEQWPSMTKLPDYKPYP 240
                ************************************************:***********

MouseCdk5       MYPATTSLVNVVPKLNATGRDLLQNLLKCNPVQRISAEEALQHPYFSDFCPP 292
RatCdk5         MYPATTSLVNVVPKLNATGRDLLQNLLKCNPVQRISAEEALQHPYFSDFCPP 292
HumanCdk5       MYPATTSLVNVVPKLNATGRDLLQNLLKCNPVQRISAEEALQHPYFSDFCPP 292
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Exhibit B
Dab1 Protein Alignment
Mouse, Rat and Human

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MouseDab1       MSTETELQVAVKTSAKKDSRKKGQDRSEATLIKRFKGEGVRYKAKLIGIDEVSAARGDKL 60
RatDab1         MSTETELQVAVKTSAKKDSRKKGQDRSEATLIKRFKGEGVRYKAKLIGIDEVSAARGDKL 60
HumanDab1       MSTETELQVAVKTSAKKDSRKKGQDRSEATLIKRFKGEGVRYKAKLIGIDEVSAARGDKL 60
                ************************************************************

MouseDab1       CQDSMMKLKGVVAGARSKGEHKQKIFLTISFGGIKIFDEKTGALQHHHAVHEISYIAKDI 120
RatDab1         CQDSMMKLKGVVAGARSKGEHKQKIFLTISFGGIKIFDEKTGALQHHHAVHEISYIAKDI 120
HumanDab1       CQDSMMKLKGVVAGARSKGEHKQKIFLTISFGGIKIFDEKTGALQHHHAVHEISYIAKDI 120
                ************************************************************

MouseDab1       TDHRAFGYVCGKEGNHRFVAIKTAQAAEPVILDLRDLFQLIYELKQREELEKKAQKDKQC 180
RatDab1         TDHRAFGYVCGKEGNHRFVAIKTAQAAEPVILDLRDLFQLIYELKQREELEKKAQKDKQC 180
HumanDab1       TDHRAFGYVCGKEGNHRFVAIKTAQAAEPVILDLRDLFQLIYELKQREELEKKAQKDKQC 180
                ************************************************************

MouseDab1       EQAVYQTILEEDVEDPVYQYIVFEAGHEPIRDPETEENIYQVPTSQKKEGVYDVPKSQPV 240
RatDab1         EQAVYQTILEEDVEDPVYQYIVFEAGHEPIRDPETEENIYQVPTSQKKEGVYDVPKSQPV 240
HumanDab1       EQAVYQTILEEDVEDPVYQYIVFEAGHEPIRDPETEENIYQVPTSQKKEGVYDVPKSQPV 240
                ************************************************************

MouseDab1       SAVTQLELFGDMSTPPDITSPPTPATPGDAFLPAPSQTLPGSADVFGSMSFGTAAVPSGY 300
RatDab1         SAVTQLELFGDMSTPPDITSPPTPATPGDAFLPAPSQTLPGSADVFGSMSFGTAAVPSGY 300
HumanDab1       SAVTQLELFGDMSTPPDITSPPTPATPGDAFIPSSSQTLPASADVFSSVPFGTAAVPSGY 300
                ********************************:*:.*****.*****.*:.**********

MouseDab1       VAMGAVLPSFWGQQPLVQQQIAMGAQPPVAQVIPGAQPIAWGQPGLFPATQQPWPTVAGQ 360
RatDab1         VAMGAVLPSFWGQQPLVQQQIAMGAQPPVAQVIPGAQPIAWGQPGLFPATQQPWPTVAGQ 360
HumanDab1       VAMGAVLPSFWGQQPLVQQQMVMGAQPPVAQVMPGAQPIAWGQPGLFPATQQPWPTVAGQ 360
                ********************:.**********:**************************

MouseDab1       FPPAAFMPTQTVMPLPAAMFQGPLTPLATVPGTNDSARSSPQSDKPRQKMGKEMFKDFQM 420
RatDab1         FPPAAFMPTQTVMPLPAAMFQGPLTPLATVPGTNDSARSSPQSDKPRQKMGKEMFKDFQM 420
HumanDab1       FPPAAFMPTQTVMPLPAAMFQGPLTPLATVPGTSDSTRSSPQTDKPRQKMGKETFKDFQM 420
                **********************************.**:*****:********** ******

MouseDab1       AQPPPVPSRKPDQPSLTCTSEAFSSYFNKVGVAQDTDDCDDFDISQLNLTPVTSTTPSTN 480
RatDab1         AQPPPVPSRKPDQPSLTCTSEAFSSYFNKVGVAQDTDDCDDFDISQLNLTPVTSTTPSTN 480
HumanDab1       AQPPPVPSRKPDQPSLTCTSEAFSSYFNKVGVAQDTDDCDDFDISQLNLTPVTSTTPSTN 480
                ************************************************************

MouseDab1       SPPTPAPRQSSPSKSSASHVSDPTADDIFEEGFESPSKSEEQEAPDGSQASSTSDPFGEP 540
RatDab1         SPPTPAPRQSSPSKSSASHVSDPTADDIFEEGFESPSKSEEQEAPDGSQASSTSDPFGEP 540
HumanDab1       SPPTPAPRQSSPSKSSASHASDPTTDDIFEEGFESPSKSEEQEAPDGSQASSNSDPFGEP 540
                ******************.****:****************************.*******

MouseDab1       SGEPSGDNISPQDGS 555
RatDab1         SGEPSGDNISPQDGS 555
HumanDab1       SGEPSGDNISPQAGS 555
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ProgramNumber:100.4 Day/Time:Saturday, Nov. 8, 4:00 PM -5:00 PM

Phospho -Dab1 is a specific biomarker for Cdk5 activation in developing and injured rodent brains.

W.Wang[1]; L.Keshvara[2]; T.Curran[2]; G.Zajic[1]; S.Jiao[1]; J.Louis[1]; C.M.Henley[1]; E.Magal[1*]

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The cytoplasmic adapter protein Dab1 is related to the Drosophila disabled gene product. It is predominantly expressed in neurons and has been shown to function downstream of Reelin in a signaling pathway that controls laminar organization in the developing mammalian brain. Cyclin-dependent kinase 5 (Cdk5) and its neuron-specific activator p35 are also required for neurite outgrowth and cortical lamination. Biochemical cross-talk exists between these two (Reelin, Cdk5) signaling pathways that control cell positioning. Cdk5 phosphorylates Dab1 on specific serine sites in vivo, thus making it a specific biomarker for Cdk5 activation. We investigated Dab1 phosphorylation in the rat brain before (controls) and after ischemia-reperfusion injury (90 minutes of middle cerebral artery occlusion (MCAO) followed by reperfusion for 2 and 6h). Embryonic wild-type and Cdk-5 knockout mouse brains were used as positive controls, since the phospho-Ser 491 Dab1 antibody has been validated in these tissues (Keshvara, et al., J. Neuroscience, 22:4869-77, 2002). P-Dab1 was present in wild type mouse brain and absent in Cdk5 knockout mouse brains. The pSer491-Dab1 antibody specificity was demonstrated by loss of signal in pre-absorbed phospho-Dab1 peptide control. Phospho-Dab1 increased 1.7 to 11-fold, respectively, in the ipsilateral cortex at 2 and 6 hours after reperfusion in the MCAO rat model. Our studies indicate: 1) Dab1 phosphorylation on serine 491 is Cdk5 activity-dependent; 2) Dab1 antibody can be used as a specific marker for Cdk5 activation; 3) Increased phosphorylation of Dab1 is observed in the MCAO rat brain, validating CDK5 activity involvement in stroke pathology.

Citation:W.Wang, L.Keshvara, T.Curran, G.Zajic, S.Jiao, J.Louis, C.M.Henley, E.Magal. Phospho -Dab1 is a specific biomarker for Cdk5 activation in developing and injured rodent brains.. Program No. 100.4. 2003AbstractViewer/ItineraryPlanner.Washington, DC: Society for Neuroscience